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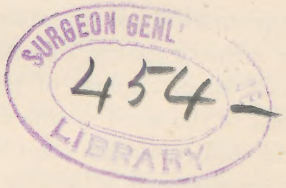

THE RELATIVE FORMATION OF PROTEOSES AND PEPTONES IN GASTRIC DIGESTION.

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(*Contributions from the Sheffield Biological Laboratory of Yale University.*)

In the digestion of proteid matter by pepsin-hydrochloric acid there are three well-defined products or classes of products, viz. acid-albumin or syntonin, albumoses or proteoses, and peptones. We look upon the first as the initial product of gastric digestion and identify it by its well-known precipitation by neutralization. In the filtrate, the albumoses can be detected by saturation of the fluid with sodium chloride and addition of acetic acid, or best by saturation with ammonium sulphate, while the true peptones (amphopeptone) are found in the filtrate from the latter precipitate, after dilution with water, by testing with cupric sulphate and potassium hydroxide[1]. True peptones are bodies not precipitated by saturation with ammonium sulphate, and are the final products of pepsin digestion.

Since the discovery of the albumoses and of their characteristics[2], frequent study of digestive mixtures has given convincing proof that in an ordinary gastric digestion of any common proteid only a comparatively small amount of peptone is formed[3]; peptonization in the true sense of the word results only from the long-continued action of pepsin-acid, and even then complete peptonization rarely if ever occurs. The albumoses or, in a broader sense, the proteoses, are to be considered as the primary products of gastric digestion, while peptones are the end products of proteolytic action, the latter being formed by the gradual

[1] Taking great care to add sufficient potassium hydroxide to decompose all of the ammonium salt present.

[2] Kühne and Chittenden, *Zeitschrift für Biologie,* Bd. xx. p. 11.

[3] See *Zeitschrift für Biologie,* Vols. 22—26; also *Studies from the Laboratory of Physiological Chemistry of Yale University,* Vols. I.—III.

hydration of the primary products; the change, however, according to our view, being a very gradual one. Obviously then, what we have often termed peptone in the past has been in reality proteose, or at the most a mixture of one or more proteoses and peptone.

With these thoughts in mind it has been the object in the experiments about to be described to obtain some positive data regarding the relative formation of albumoses and peptone in artificial gastric digestion, especially in view of the fact that certain observers claim that peptonization may result from a direct change of acid-albumin and that "propeptone" or albumose is not a necessary antecedent of peptone, or that, if so, it is quickly converted into peptone. Boas[1] even goes so far as to say that peptone alone is to be looked on as the product of the action of pepsin and acid, that acid-albumin and propeptone are simply bye products resulting from the action of acid alone, and that where active gastric juice is present there peptone only will be found as the final end product.

Obviously, we must first decide what we understand by the word peptone. All physiologists are presumably agreed that peptone is the final product of gastric digestion, but many still persist in applying the term to any or all products of gastric digestion not precipitable by neutralization, or on addition of acetic acid and saturated solution of sodium chloride. The latter test, however, will not show the presence even of deuteroalbumose, and will only incompletely precipitate proto and heteroalbumose unless the salt is present to saturation. The only certain method of separating the primary products of digestion from peptone is to saturate the neutral digestive fluid with ammonium sulphate, while hot, and even then it is sometimes difficult to remove the last trace of deuteroalbumose. A negative reaction therefore with acetic acid and saturated salt solution is no proof whatever of the absence of proteoses. Again, some physiologists claim that peptones are precipitated by saturation with ammonium sulphate and that consequently this reaction is void of significance. To this there are two answers; first, if so the precipitation is very incomplete, since in a vigorous gastric digestion the filtrate from the ammonium sulphate precipitate gives a strong biuret reaction; secondly, the ammonium sulphate precipitate (after dialysis of the ammonium salt) if redigested with a fresh gastric juice is so changed that only a portion of it is reprecipitated on again saturating the fluid with the ammonium salt,

[1] *Jahresbericht für Thierchemie für* 1887, p. 264.

and moreover the composition of the substance is likewise changed in conformity with the theory of further hydration. If then we understand peptone to be the final product of peptic action, we must admit that the substance precipitated by ammonium sulphate is an intermediate body capable of further conversion, in part at least, into true peptone.

Previous study of the individual proteoses formed in the gastric digestion of albumin, fibrin, globulin, myosin, casein and other proteids has seemingly already shown that these so-called preliminary bodies are the main products of the digestive action of pepsin, where the ferment is of moderate strength and the period of digestion not too long, say 12—24 hours, and that peptone only results from the further change of these antecedent bodies. Without doubt, the amount of true peptone may be greatly increased by increasing the strength of the pepsin solution, etc., but according to our view under no ordinary circumstances can an artificial pepsin digestion be so conducted as to have the proteoses give place entirely to peptone. There will invariably be found a residual quantity of proteoses, precipitable by saturation with ammonium sulphate.

In the present experiments, therefore, we have endeavoured to so arrange the conditions that a fair test of this theory could be made. The pepsin solutions employed were of great strength as well as purity and the digestions in many cases were continued for periods as long as six days. At the end of the specified time, the amounts of neutralization precipitate and proteoses were determined by as exact quantitative methods as possible, the values for peptone being obtained by difference. In this way, under conditions most favourable for vigorous ferment action we have endeavored to prove the accuracy of our views.

The proteid matter first employed was coagulated egg-albumin, prepared by boiling eggs in the shell for ten minutes, separating the albumin and chopping it fine. Later, blood fibrin and various other proteoses were likewise experimented with. The pepsin solutions were variously prepared, the strength of acid being 0·2 and 0·4 per cent. HCL. In every case the digestive strength of the pepsin-acid solution was determined by preliminary experiment.

Analytical Methods.

At the end of the specified time the digestions were interrupted by neutralization of the acid with a standard solution of sodium car-

bonate, the mixture warmed gently and the neutralization precipitate collected on a dried and weighed filter. After being washed with hot water until the washings gave no reaction with silver nitrate, the precipitate was finally washed with alcohol and dried at 110° C. until of constant weight.

The filtrate and washings containing the proteoses and peptone were concentrated to a small volume, made slightly acid with acetic acid, and then while hot carefully saturated with pure ammonium sulphate. In this operation care was exercised not to add an excess of the ammonium salt, but just enough to completely saturate the fluid. Finally, the completely saturated fluid was heated almost to boiling and when a film of the ammonium salt appeared on the surface of the fluid and the proteoses were collected together in a solid gummy mass, the hot fluid containing the peptone was quickly decanted, while the proteoses usually adhered tenaciously to the walls of the vessel. In some cases, however, it was found necessary to collect the precipitate on a filter, using a hot water funnel. The precipitated albumoses were then washed several times with a hot saturated solution of ammonium sulphate for the complete removal of the peptone. Owing to the gummy character of the proteose precipitate this could be accomplished only by thoroughly kneading the mass with the hot or even boiling sulphate solution. The washed precipitate was next dissolved in a little hot water and the solution carefully rinsed into a small weighed capsule, in which it was evaporated to dryness on a water-bath and then dried at 110° C. until of constant weight. The ammonium sulphate contained in the proteoses was next determined. This was accomplished by dissolving the dried mass in considerable water, filtering from any undissolved matter, adding a drop or two of hydrochloric acid and precipitating the sulphuric acid with barium chloride from a hot dilute fluid after the ordinary method[1]. From the weight of barium sulphate so obtained the ammonium sulphate was calculated[2], and the weight deducted from the weight of the proteose precipitate. Duplicate trials of this method of determining the proteoses gave closely agreeing results, thus indicating its accuracy. Deducting the combined weight of the proteose and neutralization precipitate from the weight of the dry proteid used in the digestion

[1] Generally it was found advantageous to dilute the solution up to some given volume and to use only a fraction, say one-fifth, for the determination of SO_3. This method likewise enabled us to verify the result by duplicate determinations.

[2] The ammonium sulphate employed in these separations was a pure, well crystallized salt and contained the theoretical amount of sulphuric acid, as shown by analysis.

gives the amount of true peptone formed, and probably with greater accuracy than any direct method of determination at present known. One other point needs to be noticed in this description of the method employed; viz. the occasional error introduced by the proteoses contained in the pepsin solution. In some cases this error was so small as to hardly need consideration, but we always recognized its possibility and obviated it by running a blank experiment with the pepsin and acid alone. That is to say, the same volume of pepsin-acid solution as used in the digestion was warmed at 40° C. for the same length of time as the digestive mixture itself and the proteoses, peptone, and neutralization precipitate determined and correction made accordingly.

First Experiment.

In this experiment 0·6 gram of a fairly pure dry scale pepsin, purified by dialysis, was dissolved in 800 c.c. of 0·2 per cent. hydrochloric acid. To this solution 120 grams of finely divided, coagulated egg-albumin were added and the mixture warmed at 40° C. in a closed flask. This quantity of pepsin and acid was capable of dissolving twelve times the amount of egg-albumin used, as shown by a trial experiment.

Solution of the proteid matter occurred within a few hours, but the first fraction of the fluid was not drawn off for analysis until the 42nd hour, the second fraction at the 69th hour, the third fraction at the 91st hour, while the last fraction was kept at 40° C. for 142 hours. Each fraction was the same in volume, and inasmuch as all of the albumin was dissolved before the first fraction was withdrawn, each portion contained the same amount of dissolved proteid or its products. Obviously, regard was had to the increase in the total volume of the digestive fluid from the 120 grams of albumin added.

The weight of dry albumin corresponding to one-fourth of the coagulated albumin, i.e. the amount of dry albumin dissolved in each fraction of fluid analyzed, was 3·547 grams[1].

Following are the results obtained, the percentages being calculated on the weight of dry albumin used.

Time.	Neutralization precipitate.		Proteoses.		Peptone.
42 hours	0·225 gram.	6·34 %	2·268 grams.	63·94 %	29·72 %
69 „	0·174 „	4·90 „	2·262 „	63·77 „	31·33 „
91 „	0·165 „	4·65 „	2·182 „	61·51 „	33·84 „
142 „	0·144 „	4·05 „	1·882 „	53·05 „	42·90 „

[1] The amount of dry proteid in each lot of coagulated albumin or fibrin employed was determined by drying a sample at 110° C. until of constant weight.

Second Experiment.

0·8 gram of a pure pepsin was dissolved in 800 c.c. of 0·4 per cent. hydrochloric acid and 120 grams of finely divided coagulated egg-albumin added. The mixture was warmed at 40° C. in a closed flask and the first fraction of one-fourth withdrawn at the end of 72 hours. Solution of the albumin occurred very quickly. Experiment showed that the above quantity of pepsin and acid was capable of dissolving 1600 grams of coagulated albumin. The amount of dry albumin equivalent to 30 grams of the coagulated albumin used was 3·880 grams.

Following are the results obtained;

Time.	Neutralization precipitate.		Proteoses.		Peptone.
72 hours	0·104 gram.	2·68 %	2·549 grams.	65·70 %	31·62 %
96 „	0·083 „	2·14 „	2·143 „	55·23 „	42·63 „
120 „	0·078 „	2·01 „	2·154 „	55·51 „	42·48 „
144 „	0·072 „	1·85 „	2·050 „	52·99 „	45·16 „

Third Experiment.

In this experiment, 150 grams of coagulated egg-albumin were added to an artificial gastric juice prepared by dissolving 1·0 gram of a very active pepsin in 1 litre of 0·2 per cent. hydrochloric acid and the mixture placed in a bath at 40° C. In less than two hours the entire amount of albumin was dissolved. The first fraction of fluid was withdrawn at the end of 6 hours, the second fraction at the end of 22 hours, the third fraction at the end of 28 hours, and the fourth and fifth fractions at the end of 118 hours. The fifth fraction differed from its companion in containing an additional amount of pepsin and acid. As is evident from the description, each fraction of the digestive fluid contained 0·2 gram of dry pepsin and 200 c.c. of 0·2 per cent. hydrochloric acid. The fifth fraction, however, at the end of 47 hours, had added to it 0·1 gram more pepsin and 200 c.c. more 0·2 per cent. hydrochloric acid. The fluid was then continued at 40° C., by the side of the fourth fraction, for 118 hours, differing from its companion simply in containing an additional quantity of pepsin and acid.

The amount of dry proteid (at 110° C.) equivalent to the coagulated albumin dissolved in each fraction was 3·854 grams. The results obtained are shown in the following table;

Time.	Neutralization precipitate.		Proteoses.		Peptone.
6 hours	0·2750 gram.	7·1 %	2·6926 grams.	69·8 %	23·1 %
22 „	0·1328 „	3·5 „	2·4246 „	62·9 „	33·6 „
28 „	0·1287 „	3·3 „	2·3781 „	61·7 „	34·9 „
118 „	0·0064 „	0·1 „	1·9388 „	50·3 „	49·6 „
118[1] „	0·0073 „	0·2 „	1·7308 „	44·9 „	54·9 „

In this experiment, as in the two preceding ones, the results give plain evidence of a slow and gradual formation of peptone; or in other words of a slow and gradual conversion of the first-formed proteoses into a body or bodies not precipitable by ammonium sulphate. It is furthermore evident that this slow formation of peptone is not due to lack of pepsin or acid, since the last fraction of digestive fluid in the above experiment had a large addition of both dilute acid and pepsin and was exposed to this increased proteolytic action at 40° C. for 71 hours and yet only 5 per cent. more peptone was formed. Obviously, there must be some other reason for this slow conversion of proteoses into peptone.

Fourth Experiment.

This experiment was much like the preceding, only acid of 0·4 per cent. strength was employed instead of the weaker 0·2 per cent. 150 grams of coagulated white of egg were placed in 1500 c.c. of 0·4 per cent. hydrochloric acid, to which 1·0 gram of a pure scale pepsin had been added. The pepsin was of such strength that one part would dissolve 3600 parts of coagulated egg-albumin. 30 grams of the coagulated albumin contained 3·5962 grams of dry proteid.

On placing the mixture at 40° C. the proteid matter was quickly dissolved. The fluid was divided into five equal parts, one of which was unfortunately lost.

Following are the results obtained;

Time.	Neutralization precipitate.		Proteoses.		Peptone.
47 hours	0·0701 gram.	1·9 %	2·2111 grams.	61·4 %	36·5 %
70 „	0·0469 „	1·3 „	1·8822 „	52·5 „	46·3 „
144 „	0·0081 „	0·2 „	1·7281 „	48·0 „	51·7 „
144[2] „	0·0081 „	0·2 „	1·7251 „	47·9 „	51·8 „

[1] With more pepsin and acid as described.

[2] At the end of 70 hours, 150 c.c. more of 0·4 per cent. hydrochloric acid were added to this fraction.

One point to be noticed in this experiment is that the additional amount of dilute acid introduced into the last fraction of the digestive mixture did not give rise to any increase in the formation of peptone. This is important as indicating that the conversion of the proteoses into peptone is not necessarily retarded by the presence of the products of digestion. Obviously, we cannot assert that the relative formation of proteose and peptone by natural digestion in the stomach is the same as in the action of artificial gastric juice, where the conditions are unavoidably somewhat different. But the fact that dilution of the digestive fluid with dilute acid did not give rise to any decided increase in the formation of peptone does indicate that in our experiments the lack of complete peptonization is not due, wholly at least, to accumulation of the products of digestion[1]; a fact which lends favour to the view that the chemical changes taking place in an artificial pepsin digestion are possibly not different from the changes in natural digestion.

Fifth Experiment.

The three digestions of this experiment were carried on in separate beakers. Each mixture contained 30 grams of freshly coagulated egg-albumin, 0·2 gram of the same pepsin as used in the preceding experiment and 300 c.c. of 0·4 per cent. hydrochloric acid. Each portion of coagulated albumin contained 4·1805 grams of dry proteid.

Following are the results obtained;

Time.	Neutralization precipitate.		Proteoses.		Peptone.
48 hours	0·1477 gram.	3·5 %	2·4546 grams.	58·7 %	37·7 %
72 „	0·1492 „	3·5 „	2·2844 „	54·6 „	41·7 „
408 „	0·0852 „	2·0 „	1·8437 „	44·1 „	53·8 „

In spite of the great length of time the third mixture was warmed at 40° C., viz. 17 days, only 53·8 per cent. of peptone was formed.

Sixth Experiment.

In this experiment, the proteid matter employed was blood fibrin, thoroughly washed and boiled. As in the preceding experiment, the individual digestions were carried on in separate beakers, each mixture consisting of 20 grams of boiled fibrin, 300 c.c. of 0·4 per cent. hydrochloric acid and 0·2 gram of pepsin. The ferment was capable of dissolving 3600

[1] See A. Sheridan Lea. "A comparative study of artificial and natural digestions." *Journal of Physiology*, Vol. xi. p. 226.

times its weight of coagulated egg-albumin. In view of the greater solubility of fibrin in gastric juice the digestions were not continued for so long periods as in the preceding experiments. The 10 grams of fibrin employed in each digestion contained 6·748 grams of dry proteid.

Following are the results obtained;

Time.	Neutralization precipitate.		Proteoses.		Peptone.
2 hours[1]	0·9507 gram.	14·0 %	5·0694 grams.	75·1 %	10·8 %
47 „	0·3094 „	4·5 „	4·7077 „	69·7 „	25·6 „
71 „	0·2839 „	4·2 „	4·2036 „	62·2 „	33·5 „

Seventh Experiment.

The proteid matter used in this experiment was blood fibrin, which had been thoroughly washed with cold water, boiled with water and then soaked for some months in alcohol. Prior to being digested it was again boiled with water to remove all traces of alcohol. The pepsin solution employed, was prepared by dissolving scrapings from the mucous membrane of a pig's stomach in 0·4 per cent. hydrochloric acid, purifying the solution by dialysis, etc., and finally bringing the acidity up to 0·2 per cent. Each digestive mixture contained 10 grams of the moist fibrin (equal to 3·2733 grams of dry proteid) and 200 c.c. of the acid pepsin solution.

Following are the results obtained;

Time.	Neutralization precipitate.		Proteoses.		Peptone.
25 hours	0·3175 gram.	9·7 %	1·3723 grams.	41·9 %	48·3 %
47 „	0·3565 „	10·9 „	0·9685 „	29·5 „	59·5 „
65 „	0·3190 „	9·7 „	0·9940 „	30·4 „	59·8 „

Here, we have the highest percentage of peptone formed that we have ever observed in the digestion of a natural proteid; nearly 60 per cent. The pepsin solution, however, was very strong and the proteid very easily digestible.

A consideration of the results of these several experiments obtained under varying conditions, manifestly leads us to the conclusion that the albumoses or proteoses are truly primary products of gastric digestion. That proteoses are normal products of the digestive action of both pepsin and acid, and not as Boas[2] states, of acid alone. Further,

[1] The fibrin was wholly dissolved by the pepsin-acid in less than one hour.

[2] *Jahresbericht der Thierchemie für* 1887, . 263—265.

proteoses are the antecedents of the true peptone, but evidently the conversion of the proteoses into peptone is a slow and gradual process. In no case have we been able to accomplish a digestion of either egg-albumin or fibrin without finding a large percentage of proteose in the resultant fluid. In this connection it is to be remembered that the method used by Boas for the detection of "propeptone" or albumose in his artificial digestions will not show the presence of deuteroalbumose, and hence his conclusions on this point are of little value. Further, there is no evidence whatever of the formation of peptone directly from acid-albumin; on the other hand, all of our results point to the formation of peptone from albumoses. Proteoses are to be considered therefore as necessary intermediate products in all gastric digestions; they are slowly converted into peptone, but under no ordinary circumstances will a digestive mixture be found free from these intermediate products, when properly tested. On the contrary, the percentage amount will be quite large.

In considering the results tabulated above, it must be remembered that the extent of digestive action is not fully measured by the percentage conversion of proteose into peptone. We must keep in mind that there are at least three normal proteoses formed in gastric digestion. Of these, proto and heteroproteose are first formed; then by further proteolytic action these are gradually converted into deuteroproteose, from which, by further hydration, peptone is formed. Now since the three proteoses are alike precipitated by saturation with ammonium sulphate, it follows that in the above results we have no measure for the rate of conversion of the primary proteoses into the deutero body.

Our view concerning the relative formation of these proteoses and their conversion into peptone being correct, it follows that under like conditions of time, etc. a much larger percentage of peptone will be formed from deuteroproteose than from proto or heteroproteose.

In this connection, experiments were made with pure caseoses prepared from pure casein of milk by gastric digestion; likewise with pure gluten-caseoses prepared in the digestion of gluten-casein of wheat with artificial gastric juice.

The experiments were conducted after the method already described, while the gastric juice employed was a strong solution of pepsin in 0·4 per cent. hydrochloric acid. The conditions and results are shown in the following table;

Time.	Neutralization precipitate.		Proteoses.		Peptone.
	Heterocaseose, 3·5972 grams[1].				
94 hours	0·0357 gram.	0·9 %	3·0179 grams.	83·9 %	15·1 %
	Protocaseose with some deuterocaseose. 3·6258 grams in each portion.				
72 hours	0·0212 gram.	0·5 %	2·7546 grams.	75·9 %	23·4 %
119 „	0·0117 „	0·3 „	2·4492 „	67·5 „	32·1 „
166 „	0·0081 „	0·2 „	2·3395 „	64·5 „	35·2 „
	Pure deuterocaseose. 2·9279 grams in each portion.				
69 „	0·0351 gram.	1·1 %	1·0985 grams.	37·5 %	61·2 %
137 „	0·1171 „	3·9 „	0·8717 „	29·7 „	66·2 „
	Protogluten-caseose. 4·2682 grams in each portion.				
69 „	0·0117 gram.	0·2 %	3·8984 grams.	91·3 %	8·3 %
137 „	0·0280 „	0·6 „	3·5719 „	83·6 „	15·6 „
	Protogluten-caseose with some deutero. 3·2697 grams in each portion.				
69 „	0·0090 gram.	0·2 %	2·5984 grams.	79·4 %	20·3 %
137 „	0·0238 „	0·7 „	2·3609 „	72·2 „	27·0 „

From these experiments it is plain, as already stated, that the primary proteoses, proto and hetero, are only slowly converted into peptone, since they must first pass through the intermediate stage of deuteroproteose. The deutero body on the other hand, standing next to peptone, is far more quickly and readily changed, as evidenced by the large percentage of peptone formed under exactly the same conditions as employed in the digestion of the other proteoses.

Our results then clearly indicate that the formation of peptone is a gradual process and that the greater part of the peptone formed by the action of pepsin-hydrochloric acid passes through the stage of albumose or proteose. Secondly, that at the end of the most vigorous gastric digestion a considerable part of the proteid digested will be in the form of proteose.

To how great an extent it will be necessary to modify these conclusions in applying them to the proteolytic changes of natural digestion, we cannot now say.

[1] All the substances were dried at 110° C. prior to being weighed.